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STUDIES ON FORMATION OF GAS IN SWEETENED CONDENSED MILK

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STUDIES ON FORMATION OF GAS IN SWEETENED CONDENSED MILK

By B. W. HAMMER

In foods treated in various ways for their preservation, micro-organisms that can resist conditions that the great majority of micro-organisms cannot withstand, occasionally develop and bring about undesirable changes. These changes are common in foods that have been subjected to heat and are the results of the presence of organisms that have the ability to resist temperatures that usually effectively sterilize the product in question. In sweetened condensed milk, which is presumably preserved by the addition of high percentages of sucrose, abnormal changes sometimes occur as a result of the presence of organisms that have the ability to develop in sugar concentrations that most organisms cannot withstand. The present paper deals with an outbreak of gas production in sweetened condensed milk.

HISTORICAL.

The literature on sweetened condensed milk has been reviewed by Coutts[1] and also by Andrewes[2]; from these reviews it is evident that this product apparently always contains living micro-organisms and that certain organisms are able to multiply to a certain extent in it. (See also Hammer[3] for review).

Abnormal conditions in milk put up in cans have been studied by a number of investigators, but the loose terminology used in some instances in describing the kind of milk worked with, makes it practically impossible to select with certainty all of the results obtained on sweetened condensed milk.

Pethybridge[4] examined gassy sweetened condensed milk put up in an Irish condensery and isolated two types of yeast, either one of which was capable of causing gas formation in inoculated cans. In the material studied by Pethybridge, blowing usually showed itself about six weeks after tinning. The fermented milk was normal in color, had no unpleasant taste and a faint alcoholic smell. Sugar from the batch used in the cans studied showed no yeasts. Part of the milk was condensed in one town and shipped unsugared to another where it was mixed with separated boiling milk. When the mixture was boiling, sugar was added and the condensing carried out at 40°C. The condensed milk from the auxiliary plant was found swarming with micro-organisms on four occasions. The condensed milk had 27.2% H_2O, 28.8%

[1]Coutts; Rp't. of the Local Gov't. Bd. on Pub. Health & Med. Subj., Food Rp't. 15. 1911.
[2]Andrewes; The Jour. of Path. and Bact., 18; 169. 1913.
[3]Iowa Agr. Expt. Sta. Res. Bull. 19. 1915.
[4]The Econ. Proc. Royal Dublin Soc. 1; 306. 1906.

solids not fat and 44.0% added sugar. The yeasts isolated fermented saturated solutions of sugar as sugar crystals were observed under the microscope.

Beveridge[5] studied the keeping properties of condensed milks at the temperature of tropical climates. He concluded that "the change in colour of certain kinds of condensed milks in tropical climates is presumably due to brown colour being developed by reducing sugars in solution at a certain temperature and so likely to be marked with an increase of acidity due to bacterial fermentation; the presence of iron in the ferric state also plays a part in the production. In sterile condensed milks, chiefly found among those brands which contain no added sugars, changes are not noticeable." This author states that the bacteria concerned in the increase in acidity are spore bearing bacilli which produce an acid fermentation of the proteins. He further states that, "The increase in consistency noticed in connection with the brown coloration in sweetened milks, is also due to bacillary fermentation, and some of the protein is consequently rendered insoluble."

Hunziker in his book, "Condensed Milk and Milk Powders,"[6] discusses blown condensed milk and deals with the production of gas by yeasts and by butyric acid bacteria. He states that, *"the thermal death point of all forms of yeast which have come to the attention of the writer in connection with a vast number of investigations of fermented condensed milk was below 180° F." In a later paragraph the following statement is made; **"The writer has isolated yeast from fermented sweetened condensed milk that produced vigorous gas formation in media containing as high as 85% sucrose (600 grams sucrose in 100 c. c. whey bouillon)." This author also believes that cans of sweetened condensed milk may bulge when exposed successively to excessive cold and to room temperature in the instance of cans not hermetically sealed.

At the nineteenth annual meeting of the Society of American Bacteriologists (Dec. 1917) Rogers and Clemmer[7] reported finding an organism of the aerogenes type producing gas, sometimes in quantities sufficient to burst the can, growing in condensed milk containing 40 to 45% cane sugar. The culture would not grow in milk containing 40% sugar in solution and the authors considered there was a crystallization of the sugar which reduced the amount in solution to a point permitting growth. The temperature to which the milk was heated was high enough to destroy the organism but there was abundant opportunity for infection, particularly by flies, between the pan and the can-filling machine.

[5]Jr. Roy Army Med. Corps. 22; 1. 1914.
[6]1914 *p. 156. **p. 160.
[7]Abs. of Bact. 2; 6. 1918.

HISTORY OF THE OUTBREAK.

In 1916 an outbreak of gassy fermentation in sweetened condensed milk was brought to the attention of the dairy section of the Iowa state college by a concern operating a number of condenseries in the United States; about ten batches put out at one plant were affected and seven to eight days elapsed between the canning of the milk and the development of pronounced swelling. The trouble was largely, altho not entirely, traced to milk that passed thru a certain filler in which the milk was considerably more exposed than in the other filler used in the plant. The first batch of milk developing gas was the worst of them all, altho the others showed vigorous gas formation.

The development of gas was so extensive that the cans were greatly bulged and rupturing was common, occurring usually along the seam. When pronounced swells that had not ruptured were opened, the milk and large volumes of gas foamed thru the opening for a considerable period of time; the viscous milk retained many bubbles of gas and accordingly made a surprisingly large volume of material.

The fermented milk had a yeasty odor and flavor but was not at all disagreeable and there was not the slightest evidence of putrefaction. Slides made by smearing out the fermented material and staining with Gram stain showed a number of yeasts in addition to bacteria. The yeasts were in general Gram+ and buds were quite commonly observed. When plates were poured from the fermented material, using ordinary beef extract agar, and held at 37° C., numerous colonies developed, the majority of which were bacteria. When beef extract agar to which 30% sucrose had been added was used for plating, the colonies developing at 37° C. were largely yeasts. The addition of the sucrose offered a distinct advantage in the isolation of the yeast; the ability of the yeast to develop in condensed milk indicated that strong sugar agar would not prevent its growth while, when ordinary agar was used, many of the bacteria that are present in all sweetened condensed milk were able to form colonies and thus complicate the isolation. The addition of 30% sucrose to the agar failed to prevent the growth of at least certain cocci; this would be expected since Andrewes[8] has found that cocci can increase in numbers in sweetened condensed milk.

The yeasts developing on the sucrose agar plates were picked off and streaked on slopes of the same agar or put into beef extract bouillon containing 30% sucrose. At 37° C. on the agar slopes the organisms grew readily, gave an abundant growth and commonly liberated quantities of gas from the condensation water while in the bouillon there was a heavy turbidity, much gas was

[8]See reference 2.

liberated and later there was a heavy sediment. When inoculated tubes of the sucrose bouillon that had been undisturbed for some time were jarred or when a wire was plunged into them, a cloud of gas bubbles was commonly released. Bouillon containing such a concentration of sucrose that crystals were forming at the bottom gave a heavy growth with the usual gas formation when inoculated with the organism, so it seems evident that the growth of the organisms can not be inhibited by sucrose alone.

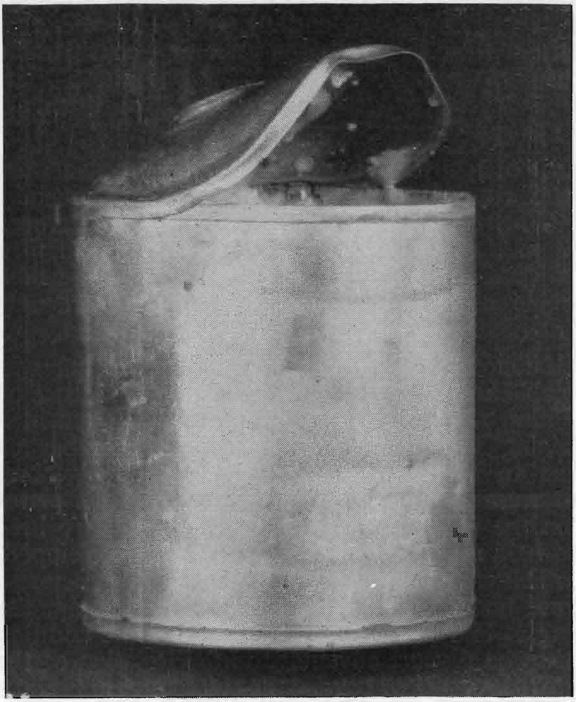

Fig. 1. Artificially infected can blown open

Inoculation experiments were then undertaken in an effort to prove the causal relationship of the organisms isolated to the trouble under consideration. The brand of condensed milk in which the fermentation was observed (Brand A) was not available on the local market so one of the other brands (Brand B) was used. A small portion of one of the ends of a can was treated with HC1 and after a short time a flamed piece of steel was driven thru the tin in this area; organisms, either from sucrose agar or sucrose bouillon were then introduced and the opening soldered shut.

The introduction of organisms thru an opening small enough to be readily soldered shut offered some difficulty and the most satisfactory method found was to use a sterilized pipette made by drawing out a piece of glass tubing with which it was possible to get the organisms from a bouillon culture or from a suspension of an agar growth deep down into the milk. Some little difficulty was encountered also by the milk oozing out thru the opening made in certain cans and when this happened satisfactory soldering was practically impossible; this nearly always occurred when the cans were dented.

A considerable number of inoculation experiments were made at 37° C. with Brand B but the results were in general negative, altho later positive results were occasionally secured. A number of cans of Brand A were then obtained and here inoculation experiments at 37° C. were usually successful; the milk in the cans fermenting after artificial inoculation had the same odor and flavor as that sent in for examination, yeast cells could be demonstrated in it by microscopical examination, and by plating methods, using agar plus 30% sucrose, a yeast could be isolated which was apparently the same as that isolated from the original milk.

At a considerably later date the same sort of a fermentation was again experienced by the concern sending in the abnormal cans, altho in this instance only a few hundred cases were involved. Cans from this lot were sent to the laboratory and an examination showed the same odor and flavor as in the first lot; similar microscopical findings were also secured and when the milk was plated out on beef extract agar plus 30% sucrose a yeast was readily isolated which was apparently the same as that found in the first outbreak. Inoculation experiments at 37° C. also showed the ability of this yeast to cause a swelling in normal cans of Brand A.

In the inoculation experiments at 37° C. the swelling of the cans was first evident in from 2 to 6 days and in general it progressed quite rapidly. As a usual thing the bulged cans were soon removed from the incubator and held at a lower temperature in order to prevent their bursting. A few cans were allowed to burst to show definitely that the organisms could produce sufficient gas to accomplish this; the appearance of one of these ruptured cans is shown in figure 1 while figure 2 shows a greatly bulged but unbroken can.

Because the yeast isolated was capable of growing in the strong sugar concentration prevailing in condensed milk, it was thought advisable to inoculate it into cans of syrups. Three different kinds of syrup were used and in each there was some swelling of the can after a few days at 37° C. at which time the cans were removed to a lower temperature to prevent their bursting.

The important thing from a practical standpoint is the source of the infection. In the first instance of the fermentation the spoiled milk was largely traced to one of the two fillers in operation in the plant, as has already been stated, and this seems to indicate that the cans were not a source of the trouble.

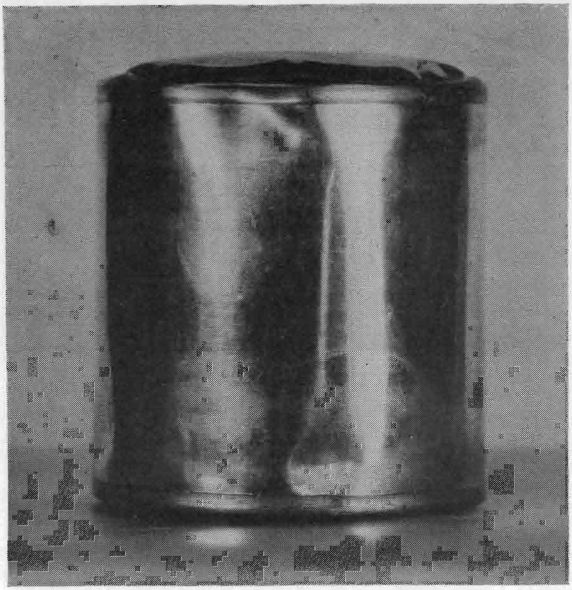

Fig. 2. Artificially infected can greatly bulged.

Because the troublesome filler was considerably more exposed than the other it is possible that the infection was air-borne, but this does not seem very probable. The most logical explanation seems to be that there was a small piece of piping, or something of that sort, that was unintentionally overlooked during the careful cleaning to which the machines and piping were subjected.

A small amount of scale from one of the pipes thru which the condensed milk passed was examined for the yeast causing the fermentation, but it was not found. A number of samples of sugar were likewise examined but these were also free from the yeast in question. From one of the samples of sugar, however, a yeast was isolated that was capable of growing and producing gas in a 30% solution of sucrose in bouillon. When this organism was inoculated into sweetened condensed milk it failed to bring about the development of gas.

From this and a number of other experiences it seems that gas-forming yeasts capable of growing in a 30% solution of sucrose in bouillon but unable to grow in sweetened condensed milk are fairly common. It is not probable that they are of any practical importance from the standpoint of the sweetened condensed milk industry but their occurrence and distribution in such materials as sugar should be further investigated.

The presence of cans that fail to ferment in a batch of milk, the most of which spoils when canned, is presumably due to a light infection. It is extremely improbable that each can of milk of a given lot would be infected unless the troublesome organism is present in quite large numbers. From the general situation existing in the condenseries it seems that the infection of the milk is by no means heavy under ordinary conditions.

The failure of certain cans to show the development of gas when inoculated with the yeast causing the trouble is very probably due to the composition of the milk. While the sugar content has a great deal to do with keeping down the growth of micro-organisms in sweetened condensed milk, the fact that the yeast which was isolated is capable of growing in a saturated sugar solution indicates that there is something else that may be a factor in restricting the growth of organisms. The brand in which it was impossible to cause blowing in the early inoculations is a brand having a very high content of milk solids and one of the theories that seems probable is that milk solids are of a great deal of importance in keeping down the growth of organisms. Inoculation experiments with a number of brands have failed to substantiate this theory, but they were entirely too few to justify definite conclusions; the variations in the composition of brands as they appear on the market complicate work of this sort. An attempt was made to prepare milks from skim milk powder, sucrose and water for the purpose of studying the influence of both milk solids and sucrose but the results were not satisfactory.

DESCRIPTION OF ORGANISM.

The yeast isolated from blown cans of sweetened condensed milk has been studied morphologically, culturally and biochemically with the following results:

MORPHOLOGY.

Form. The organism in general was oval in form. Occasionally a cell with a round appearance was seen but this was presumably the result of viewing it from the end.

Size. In preparations made from both young and old sucrose (30%) agar slopes at either 37° C. or room temperature the great majority of the organisms were from 2.3 to 2.9 microns in length and from 1.2 to 1.8 microns in width. The organisms were of approximately the same size when grown in sweetened condensed milk.

Arrangement. The organisms were usually lying free or showed a single bud. Apparently the buds did not remain attached to the mother cell for any considerable period.

Spore Formation. Spore formation was not observed. See discussion following this description.

Staining Reaction. The organism stained readily with the ordinary stains. It was Gram+ with usually some Gram— cells present; in general the percentage of Gram— cells increased with age but even in very old cultures some Gram+ cells were found.

CULTURAL CHARACTERISTICS.

Sucrose (30%) Agar Slope. After 24 hours at 37° C. growth was evident as small, somewhat transparent, smooth-edged, slightly raised, non-viscid colonies. With age, the growth increased in amount and became less transparent and in certain areas of the slope the colonies grew together. The same type of growth was secured at room temperature as at 37° C. altho the development was not as rapid.

Whey Agar Slope. Growth apparently did not occur without the addition of sugar which the organism could ferment.

Beerwort Agar Slope. The same general type of growth was secured on beerwort agar as on sucrose agar but growth apparently was not quite as rapid.

Sucrose (30%) Agar Stab. After 24 hours at 37° C. sucrose (30%) agar stabs showed a great deal of splitting as a result of the formation of gas, and in certain cases a mass of agar was forced up against the stopper. Gas formation continued with age and resulted in a still more extensive breaking up of the medium.

Agar Plate Colony. At 37° C. colonies that were somewhat transparent were evident after 24 hours. Growth increased for several days at which time the surface colonies were white, round, smooth-edged, non-viscid and up to 2 m. m. or more in diameter while the subsurface colonies were white, ellipsoidal, smooth-edged, non-viscid and up to 1 m. m. in their long diameter. With an increase in age there was a decrease in the transparency. Under the low power the individual yeast cells could be readily seen along the edge of the surface colonies. Growth was of essentially the same type at room temperature but was much slower.

Whey Gelatine Stab. Growth apparently did not occur without the addition of sugar which the organism could ferment.

Bouillons. Growth was not observed except in the presence of sucrose, glucose or fructose altho in a few instances there was apparently some slight growth in the presence of galactose. Where growth occurred it consisted of a turbidity, usually evident after 24 hours at 37° C. and somewhat later at room temperature, which soon cleared leaving only a heavy sediment.

Potato. There was no evidence of growth at either 37° C. or room temperature.

Dunham's Solution. There was no evidence of growth at either 37° C. or room temperature.

Uschinsky's Solution. There was no evidence of growth at either 37° C. or room temperature.

Litmus Milk. There was no evidence of growth in litmus milk even after 33 days at 37° C. altho the inoculated milk still contained living organisms since growth was readily secured on sucrose agar slopes.

Plain Milk. There was no evidence of growth in inoculated milk even after long periods of incubation.

BIO-CHEMICAL FEATURES.

Gas Formation.—Gas was produced in bouillon containing glucose, fructose or sucrose but no evidence of gas formation was secured in bouillon containing galactose, maltose, lactose, mannitol or salicin. Large amounts of gas were produced in sweetened condensed milk.

Oxygen Relation.—The organism was facultative.

Reaction Change. Where there was gas formation in sugar bouillon there was an acidity produced that was roughly equivalent to from 2 to 3% n/1 acid.

Odor. All cultures that showed an abundant growth had a very yeasty odor.

Alcohol Production. Some of the data secured on the amount of alcohol produced are presented in table 1.

TABLE 1—ALCOHOL PRODUCTION.

Material	Conditions of Incubation	Percent Alcohol by Weight*
Original spoiled sweetened condens'd	Unknown	.95
Bouillon plus 30% sucrose	13 days at 37°C.	3.94
" " 40% "	" " " "	3.35
" " 50% "	" " " "	1.75

*Alcohol determined by distilling and calculating from the Sp. Gr. of the distillate.

COMPARISON OF THE ORGANISMS STUDIED WITH OTHER ORGANISMS ISOLATED FROM BLOWN SWEETENED CONDENSED MILK.

A comparison of the abnormal sweetened condensed milk studied with that investigated by others leads to the following consideration:

In the outbreak described by Pethybridge the time elapsing between the canning and blowing of the milk was very long, but this may have been due to the temperature prevailing in the place of storage, since at 37° C. with inoculation only a few days were required for the cans to show gas formation. The organisms isolated by Pethybridge were of a different shape than the organism studied at this station, the one being longer and narrower, and the other being spherical; the shape may have been influenced by the medium and undoubtedly by other factors and it seems safest to assume that it is impossible to determine whether or not one of the organisms described by Pethybridge is identical with the yeast isolated in the present investigation because of the meager description given by that author. The organism referred to by Hunziker[9] has not been described in the literature, while Rogers and Clemmer in their investigation found an organism of the aerogenes type responsible for the blowing.

Spores were never observed with the budding organism isolated from sweetened condensed milk but no effort was made to stimulate spore production. The formation of spores was not studied because the usefulness of attempting to employ sporulation in a diagnostic way with yeasts is in doubt. Hunter,[10] in considering this question, points out that observations of different investigators on sporulation for the same yeast do not agree and also

[9]Letter from Prof. Hunziker.

[10]Hunter, O. W. A Lactose Fermenting Yeast Producing Foamy Cream. Jour. Bact. 3, 293. 1918.

that Hansen states that the Torula may be only a temporary stage of development of yeasts and has demonstrated that spore production is not a stable factor for he has been able to produce an asporogenic race of Saccharomyces by varying the conditions of cultivation. The uncertainty of the technique used in determining whether or not sporulation occurs is another objection that decidedly limits the value of spore formation as a means of classification.

It seems best, because of the importance of blowing in sweetened condensed milk, to suggest a name for the budding organism isolated and *Torula lactis-condensi* is accordingly proposed; the generic name Torula* is used tentatively and with the idea that a satisfactory classification of the budding organisms will probably result in a change. It is to be expected that the yeasts causing blowing in sweetened condensed milk are not all alike because organisms growing in strong sugar solutions are quite common and these are certainly not all the same type, but a definite statement on this point will only be possible after a comparison of the organisms isolated from a number of different outbreaks. The description presented is intended as a starting point for such a study.

SUMMARY.

An outbreak of gas formation in sweetened condensed milk was studied and found to be due to a budding organism. Inoculation experiments, using normal cans of milk, proved the causal relationship of the organism to the condition studied.

The organism which was named *Torula lactis-condensi* is described and thus will provide a starting point for an investigation to determine whether or not more than one type of budding organism is concerned with the formation of gas in cans of sweetened condensed milk.

Yeasts capable of growing in 30% solutions of sucrose can be commonly isolated but most of these are unable to grow in sweetened condensed milk.

It seems that there is a variation among the different brands of condensed milk in their susceptibility to fermentation with the yeast studied. This may be due to differences in the composition and since the yeast can grow in a saturated sucrose solution it is possible that the milk solids play a part in keeping down growth.

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*Atelosaccharomyces or Cryptococcus would be preferred by some authors. See Anderson. Yeast-like Fungi of the Human Intestinal Tract. Jour. Inf. Dis. 21; 374-375. 1917.